II. REMARKS

Claims 80-118 are pending and under consider in the present application. Claim 119 has been added. Claims 117 and 118 have been amended.

No new matter has been added with the amendments and newly added claim. The amendments to claims 117 and 118 are non-substantive changes. Newly added claim 119, which recites that the system further comprises excitation light that is in contact with the reagent mixtures, is supported in the specification for example, at page 53, lines 11-25. Upon entry of the present amendment, claims 80-119 will be pending and under consideration.

Rejections under 35 U.S.C. § 112, Second Paragraph

Claims 117 and 118 stand rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention. Applicants respectfully traverse the rejection.

The Office Action alleges that claims 117 and 118 are indefinite for failing to disclose a positive limitation in the claim in reciting the phrase "can be." Claim 117 has been amended to recite that the source of excitation light is positioned such that the living cells *are* excited at an excitation angle. Claim 118 recites that the detector is positioned such that emitted light *is* collected at about 12.5 degrees from the excitation angle. Accordingly, Applicants respectfully request withdrawal of the rejection of claims 117 and 118 under 35 U.S.C. § 112, second paragraph

Prior Art Rejections

As a preliminary matter, the Office Action indicates that in view of Applicant's amendment, the rejection of claims 80-104 and 113-116 under 35 U.S.C. § 103(a) as being unpatentable over Akong et al. (U.S. Pat. No. 6,372,183) in view of Cubbage et al. (U.S. Pat. No. 5,582,892) is withdrawn. Applicants respectfully request acknowledgement that the rejection of claims 80-104 and 113-116 under 35 U.S.C. § 103(a) as being unpatentable over Dunlay et al. (U.S. Pat. No. 5,989,835) in view of Cubbage et al. (U.S. Pat. No. 5,582,982) is

withdrawn. This rejection was included in the Office Action mailed June 18, 2002, but not in the Office Action mailed March 11, 2003.

Claims 80-118 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Dunlay et al. (U.S. Pat. No. 5,989,835) in view of Cubbage et al. (U.S. Pat. No. 5,582,982) and in further view of Li et al (U.S. Pat. No. 5,637,505). Applicants respectfully traverse the rejection.

To establish a *prima facie* case of obviousness there must be some suggestion or motivation in the prior art to make the claimed invention, there must be a reasonable expectation of success, and the prior art reference must teach or suggest all of the claim limitations. MPEP § 2142; In re Vaeck, 947 F.2d 488, 20 USPQ2d, 1438 (Fed. Cir. 1991).

The Office Action asserts that Dunlay et al. disclose a system for high throughput screening of large numbers of compounds that affect biological functions of cells. Furthermore, the Office Action asserts that the system of Dunlay et al. includes an aqueous solution of cells and automatically adds photon producing agents such as fluorophores and photon reducing agents such as dyes to cells of a multiwell plate. The Office Action asserts that Cubbage et al. disclose compositions for use in fluorescent assays that include a photon producing agent that is incubated with an aqueous solution containing living cells and a photon reducing agent that has a wavelength range that includes the emission wavelength of the photon producing agent. Furthermore, the Office Action asserts that Cubbage et al. specifically teach that it is desirable for the photon reducing agent to remain in the solution in which cells are suspended. Finally, the Office Action asserts that Li et al. disclose Tartrazine dyes and teach using the dyes in combination with other dyes in dye-based reference materials that are formulated for clinical testing (claims 105-112), therefore allegedly teaching that the second reagent of the present invention can include at least two photon reducing agents.

Applicants respectfully assert that claims 80-118 are not rendered obvious by Dunlay et al. in view of Cubbage et al. and Li et al. because these references do not teach or suggest all of the claimed limitations. Regarding Dunlay et al., the present Office Action (page 5, first full

C

paragraph), like the prior Office Action, acknowledges that this reference does not teach that the photon reducing agent is substantially impermeant to a plasma membrane of a living cell, or that the photon reducing agent reduces light emitted from the aqueous reagent mixture by at least 10%.

These omissions of Dunlay et al. go to a distinction of the present invention over the art noted in the present specification. In the passage cited in the Office Action, Dunlay et al. state that a variety of ways are available to measure fluorescence, including by measuring quenching of fluorescence. As discussed in the pending specification, the present invention overcomes solution fluorescence that often interferes with cell-based fluorescence assays by including at least one photon reducing agent (Page 4, lines 11-19) that preferably does not cross the cell membrane (Page 20, lines 24-25). Dunlay et al. in the passage cited in the Office Action simply refer to known methods for measuring fluorescence. As stated in the present specification, these known methods suffered from the problem of background fluorescence. Dunley et al. do not teach using a photon reducing agent to reduce light emitted from an aqueous reagent mixture. Furthermore, as acknowledged in the Office Action, Dunley et al. is silent as to the photon reducing agent being substantially impermeant to a plasma membrane of a living cell and that the photon reducing agent reduces light emitted from the aqueous reagent mixture by at least 10%. These elements are related to the unexpected result disclosed in the pending specification that solution fluorescence can be reduced by including a photon reducing agent that is substantially impermeant to a plasma membrane in an aqueous reagent mixture.

Regarding Cubbage et al., as asserted in the Response mailed December 30, 2002, Applicants reassert that this reference does not provide the elements missing in Dunlay et al. Cubbage *et al.* reports the use of background-reducing compounds for use with analytical methods to measure the number of probe molecules bound to a biological entity, to determine the amount of target molecules in that entity. In the methods disclosed in Cubbage *et al.*, the background reducing compounds are used to reduce the non-specific binding of probe molecules on the surface of and *inside* the biological entity by competing with non-specific binding sites.

Therefore, Cubbage et al. teaches that a background reducing dye should be membrane permeable, not substantially impermeant to a plasma membrane, as recited in the claimed invention.

The Office Action asserts that Cubbage et al. specifically teach that in assays it is desirable for the photon reducing agent to remain in the solution in which the cells are suspended (i.e. outside of the membrane of cells), allegedly suggesting the advantage of impermeability of photon reducing agents to cell membranes. Teaching that a photon reducing agent remains in solution is not the same as teaching that the photon reducing agent is substantially impermeant to a plasma membrane. Cubbage et al. teach that "the cells or viruses should remain in the probe-free solution comprising the background reducing compound for a time great enough *to allow them to absorb the background-reducing compound*" (emphasis added) (Col. 4, lines 12-17). Furthermore, Cubbage et al. teach that "the background reducing compound will diffuse onto and *into* the biological entity" (emphasis added) (Col. 3, lines 41-51, Cubbage et.). Therefore, Applicants respectfully assert that Cubbage et al. does not suggest the use of a photon reducing agent that is substantially impermeant to a plasma membrane.

The Office Action asserts that Li et al. teach inherently membrane impermeable dyes such as Tartrazine and patent blue having known absorption maxima that can be combined with other dyes and/or other photon producing agents to exhibit distinct absorption wavelengths. Since neither Cubbage et al. nor Dunlay et al. teach or suggest that the photon reducing agent is substantially impermeant to a plasma membrane of a living cell, Applicants reassert that it would not have been obvious to incorporate the dyes in the reference material taught by Li et al. into the system taught by Dunlay et al. Furthermore, since Cubbage et al. teach that the background reducing compound must be absorbed by the cell, it is improper to rely on Li et al., for a teaching of membrane impermeant dyes, which would conflict with the teaching of Cubbage et al.

Furthermore, it is improper to rely on Li et al. for an obviousness rejection because it is nonanalogous art to the claimed invention and to the other art relied upon in the rejection. The pending claims recite a system for fluorescence assays. The primary reference, Dunlay et al.,

teaches a system for cell-based fluorescence screening and the secondary reference, Cubbage et al., teaches compounds for fluorimetric flow cytometric assays. Li et al., on the other hand, provide quality control reference material that reflect the absorbance pattern of certain hemoglobin fractions of whole blood for use in absorbance-based oximeters (Li et al., Col. 1, lines 57-62). Therefore, a person of ordinary skill in the art would not reasonably expect to solve the problem of background fluorescence in a fluorescence assay system using a reference standard for an absorbance-based oximeter. Accordingly, Li et al. is non-analogous art (See MPEP 2131.01 citing In re Clay, 966 F.2d 656 (Fed. Cir. 1992) (holding art for reducing the permeability of underground hydrocarbon bearing formations using a gel, nonanalogous to a claimed invention directed at a gelling process for preventing product loss of a refined liquid hydrocarbon)). Since Li et al. is non-analogous art, it is improper to rely on Li et al. in a rejection against the pending claims and improper to combine Li et al. with Cubbage et al. and Dunlay et al. because Li et al. pertains to nonanalogous art.

Regarding dependent claims 114 to 116, as acknowledged on pages 7 and 8 of the Office Action, that Dunlay et al., Cubbage et al., and Li et al. fail to teach that the steady state concentration of the photon reducing agent within living cells is less than 50%, 30%, and 10% of the concentration of the photon reducing agent outside the living cells, as recited in claims 114 to 116, respectively. However, the Office Action asserts that such teachings are obvious optimum workable ranges of the compositions disclosed by the prior art by normal optimization procedures.

Applicants respectfully disagree with this conclusion. As disclosed in the present application, these steady state concentrations are directed to the stated purpose of minimizing solution fluorescence. Even if these were concentrations that could be achieved by optimization procedures, one of ordinary skill in the art would not have been motivated to perform such experiments because Dunlay et al., Cubbage et al., and Li et al. are silent as to using a substantially membrane impermeant photon reducing agent to reduce light emitted from an aqueous mixture in a fluorescent assay.

C

Therefore, Applicants respectfully assert that claims 80-118 are not obvious under 35 U.S.C. § 103(a) over Dunlay et al. (U.S. Pat. No. 5,989,835) in view of Cubbage et al. (U.S. Pat. No. 5,582,982) and in further view of Li et al (U.S. at. No. 5,637,505). Accordingly, Applicants respectfully request withdrawal of the rejection.

Claims 80 to 116 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Akong et al. (U.S. Pat. No. 6,372,183) in view of Cubbage et al. (U.S. Pat. No. 5,582,982) and in further view of Li et al. (U.S. Pat No. 5,637,505). Applicants respectfully traverse the rejection.

The Office Action asserts that Akong et al. disclose a computer controlled measurement system for a fluorescence assay of activity in living cells that includes a light source and filters for excitation, and a detector. Furthermore, the Office Action asserts that the system of Akong et al. automatically adds photon producing agents such as fluorescent indicators and photon reducing agents such as dyes to cells of a multiwell plate. The Office Action asserts that Cubbage et al. disclose compositions for use in fluorescent assays that include a photon producing agent that is incubated with an aqueous solution containing living cells and a photon reducing agent that has a wavelength range that includes the emission wavelength of the photon producing agent. Furthermore, the Office Action asserts that Cubbage et al. specifically teach that it is desirable for the photon reducing agent to remain in the solution in which cells are suspended. Finally, the Office Action asserts that Li et al. disclose Tartrazine dyes and teach using the dyes in combination with other dyes in dye-based reference materials that are formulated for clinical testing (claims 105-112), therefore allegedly teaching that the second reagent of the present invention can include at least two photon reducing agents.

Applicants respectfully assert that claims 80-118 are not rendered obvious by Akong et al. in view of Cubbage et al. and Li et al. because these references do not teach or suggest all of the claimed limitations. Regarding Akong et al., the present Office Action (page 10, lines 1-4,

and page 14, second full paragraph), like the prior Office Action, acknowledges that this reference does not teach that the photon reducing agent is substantially impermeant to a plasma membrane of a living cell, or that the photon reducing agent reduces light emitted from the aqueous reagent mixture by at least 10%. This reference does not even mention photon reducing agents. These omissions of Akong et al. go to a distinction of the present invention over the art noted in the present specification. As discussed in the pending specification, the present invention overcomes solution fluorescence that often interferes with cell-based fluorescence assays by including at least one photon reducing agent (Page 4, lines 11-19) that preferably does not cross the cell membrane (Page 20, lines 24-25).

Furthermore, Akong et al. does not teach methods for reducing background fluorescence, other than teaching that fluorescence should be measured *before adding reagents*, to obtain a background value that can be used in post-reagent data processing (column 3, lines 50-59). This teaching is incompatible with the present invention because if background values are measured using the system of the present invention before adding any reagents, including a reagent with a photon-reducing agent, background values so obtained would not be accurate.

Regarding Cubbage et al., as asserted in the Response mailed December 30, 2002, Applicants reassert that this reference does not provide the elements missing in Akong et al. Cubbage *et al.* reports the use of background-reducing compounds for use with analytical methods to measure the number of probe molecules bound to a biological entity, to determine the amount of target molecules in that entity. In the methods disclosed in Cubbage *et al.*, the background reducing compounds are used to reduce the non-specific binding of probe molecules on the surface of and *inside* the biological entity by competing with non-specific binding sites. Therefore, Cubbage et al. teaches that a background reducing dye should be membrane permeable, not substantially impermeant to a plasma membrane, as recited in the claimed invention.

The Office Action asserts that Cubbage et al. specifically teach that in assays it is desirable for the photon reducing agent to remain in the solution in which the cells are suspended

C

(i.e. outside of the membrane of cells), allegedly suggesting the advantage of impermeability of photon reducing agents to cell membranes. Teaching that a photon reducing agent remains in solution is not the same as teaching that the photon reducing agent is substantially impermeant to a plasma membrane. Cubbage et al. teach that "the cells or viruses should remain in the probe-free solution comprising the background reducing compound for a time great enough *to allow them to absorb the background-reducing compound*" (emphasis added) (Col. 4, lines 12-17). Furthermore, Cubbage et al. teach that "the background reducing compound will diffuse onto and *into* the biological entity" (emphasis added) (Col. 3, lines 41-51, Cubbage et al.). Therefore, Applicants respectfully assert that Cubbage et al. does not suggest the use of a photon reducing agent that is substantially impermeant to a plasma membrane.

The Office Action asserts that Li et al. teach inherently membrane impermeable dyes such as Tartrazine and patent blue having known absorption maxima that can be combined with other dyes and/or other photon producing agents to exhibit distinct absorption wavelengths. Since neither Cubbage et al. nor Akong et al. teach or suggest that the photon reducing agent is substantially impermeant to a plasma membrane of a living cell, Applicants reassert that it would not have been obvious to incorporate the dyes in the reference material taught by Li et al. into the system taught by Akong et al. Furthermore, since Cubbage et al. teach that the background reducing compound must be absorbed by the cell, it is improper to rely on Li et al., for a teaching of membrane impermeant dyes, which would conflict with the teaching of Cubbage et al.

Furthermore, it is improper to rely on Li et al. for an obviousness rejection because it is nonanalogous art to the claimed invention and to the other art relied upon in the rejection. The pending claims recite a system for fluorescence assays. The primary reference, Akong et al., teaches a system for cell-based fluorescence screening and the secondary reference, Cubbage et al., teaches compounds for fluorimetric flow cytometric assays. Li et al., on the other hand, provide quality control reference material that reflect the absorbance pattern of certain hemoglobin fractions of whole blood for use in absorbance-based oximeters (Li et al., Col. 1, lines 57-62). Therefore, a person of ordinary skill in the art would not reasonably expect to solve

C

the problem of background fluorescence in a fluorescence assay system using a reference standard for an absorbance-based oximeter. Accordingly, Li et al. is non-analogous art (See MPEP 2131.01 citing In re Clay, 966 F.2d 656 (Fed. Cir. 1992) (holding art for reducing the permeability of underground hydrocarbon bearing formations using a gel, nonanalogous to a claimed invention directed at a gelling process for preventing product loss of a refined liquid hydrocarbon)). Since Li et al. is non-analogous art, it is improper to rely on Li et al. in a rejection against the pending claims and improper to combine Li et al. with Cubbage et al. and Akong et al.

Regarding dependent claims 114-116, the Office Action acknowledges that Akong et al., Cubbage et al., and Li et al. fail to teach that the steady state concentration of the photon reducing agent within living cells is less than 50%, 30%, and 10% of the concentration of the photon reducing agent outside the living cells, as recited in claims 114 to 116, respectively (See page 11, first full paragraph of Office Action). However, the Office Action asserts that such teachings are obvious optimum workable ranges of the compositions disclosed by the prior art by normal optimization procedures.

Applicants respectfully disagree with this conclusion. As disclosed in the present application, these steady state concentrations are directed to the stated purpose of minimizing solution fluorescence. Even if these were concentrations that could be achieved by optimization procedures, one of ordinary skill in the art would not have been motivated to perform such experiments because Akong et al., Cubbage et al., and Li et al. are silent as to using a substantially membrane impermeant photon reducing agent to reduce light emitted from an aqueous mixture in a fluorescent assay.

Therefore, Applicants respectfully assert that claims 80-118 are not obvious under 35 U.S.C. § 103(a) over Akong et al. (U.S. Pat. No. 5,989,835) in view of Cubbage et al. (U.S. Pat. No. 5,582,982) and in further view of Li et al (U.S. at. No. 5,637,505). Accordingly, Applicants respectfully request withdrawal of the rejection of Claims 80 to 116 under

35 U.S.C. § 103(a) as being unpatentable over Akong et al in view of Cubbage et al. and in further view of Li et al.

Regarding newly added claim 119, the cited art either alone or in combination does not teach a system for fluorescence assays that includes a source of excitation light in contact with an aqueous reagent mixture having a plurality of cells, a first reagent that includes a photon producing agent, and a second reagent that includes a substantially membrane impermeant photon reducing agent. This aspect of the invention has the advantage, especially in a high throughput assay format, of providing a system that does not require a washing step (See present application page 53, lines 11-21). Therefore, the system can be used to perform homogeneous assays.

Regarding Dunlay et al. and Akong et al., since the Office Action acknowledges that Dunlay et al. and Akong et al. do not teach a photon reducing agent that is substantially impermeant to a plasma membrane, the Office Action impliedly acknowledges that Dunley et al. and Akong et al. do not teach this aspect of the invention. Li et al. is not related to fluorescent systems, therefore it does not teach this aspect of the invention. Cubbage et al. is also silent as to this aspect of the invention. In fact, Cubbage et al. teach away from this aspect of the invention. For example, at column 2, lines 50-56, Cubbage et al. teach that the biological entity (e.g., cell) is removed from the fluorescent probe solution before the biological entity is exposed to light absorption wavelengths. Furthermore, Cubbage et al. teaches that in preferred embodiments their invention includes a washing step between the step wherein the biological entity is removed from the probe solution and the step wherein the biological entity is exposed to light. (Col. 4, lines 23-25).

C

The Examiner is invited to contact Applicants' undersigned representative if there are any questions relating to this application. Accompanying this response is a petition for two-months extension of time and the required fee. The Commissioner is authorized to charge any additional fees that may be required, or credit any overpayments, to Deposit Account No. 50-1355.

Respectfully submitted,

Dated: June 11, 2003

Lisa A. Haile, Ph.D.
Registration No. 38,347
Telephone: (619) 677-1456
Facsimile: (619) 677-1465

GRAY CARY WARE & FREIDENRICH LLP
4365 Executive Drive, Suite 1100
San Diego, California 92121-2133
USPTO Customer Number 28213

C